# DEVELOPMENT AND CHROMOSOME MECHANICS IN NEMATODES: RESULTS FROM ML-1

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## ABSTRACT

A subset of the *Caenorhabditis elegans* nematodes flown aboard Biorack on IML-1 was analyzed for the fidelity of development and the mechanics of chromosomes at meiosis. To assess meiosis, mutant worms marked at two linked or unlinked loci were inoculated as heterozygous hermaphrodites and allowed to self fertilize. Mendelian segregation ratios and recombination frequency were measured for offspring produced at 1 XG or in microgravity. To assess development, worms and embryos were fixed and stained with the DNA dye, DAPI, or antibodies specific for antigens expressed in germ cells, pharyngeal and bod y wall muscles, and gut cells. The distribution of cytoplasmic determinants, cell nuclei counts and positions were scored 10 assess symmetry relations and anatomical features,

## INTRODUCTION

The International Microgravity Laboratory # 1 SpaceLab mission using the ESA Biorack facility provided a capability for observing animal development as a function of gravity and for exposing sensitive germ cells to the natural radiation environment of Space. The nematode *Caenorhabditis elegans* was used for both purposes as part of an experiment entitled “Genetic and Molecular Dosimetry of HZE Radiation”; it is also known as “US-1” or "Radiat" in Biorack and IML- 1 mission documentation. The principal objective of the experiment was to measure the mutagenic activity of cosmic rays on animal cells, but the fidelity of development and assessments of normal chromosome behavior were secondary objectives and also served as indirect gravity controls for the radiobiology measurements. The development studies are the subject of this paper. The radiobiology experiments are described in paper F2.7-M.2.02 of this congress /1/.

## HARDWARE DESCRIPTION AND METHODS

### Hardware

The ESA Biorack is a miniaturized biological laboratory which takes the form of a Spacelab rack and provides a variety of services which include: incubators, 1 XG centrifuges, coolers, glove box and microscope /2/. Ancillary equipment provides passive temperature control to and from orbit in the shuttle mid-deck (PTCUs). All experiments utilizing Biorack are housed in one of two types of standardized boxes called Type I or Type II containers. Type I containers were used for this portion of the *Radiat* experiment. Each Type 1 is an anodized aluminum box of approximately 20 x 40 x 80 mm internal dimensions with spring latches and alignment flanges to interface with Biorack centrifuges and storage racks. The *Radiat* development experiments used Type I’s placed in Biorack incubator A at 22°C on the 1 XG centrifuge or at OXG on the side racks. Additional Type I’s were placed in a Nomex® nylon belt with pouches attached to the Spacelab tunnel (a low shielding area selected to maximize radiation exposure) via Velcro® patches. For the latter samples temperature control was provided by the overall Spacelab/Shuttle life support system and varied between 20 and 26 degrees C. as reported by an automatic temperature recorder which accompanied the samples.

Inside the Type I containers were 4 or 8 lexan tubes with silicone stoppers each containing a fixed volume of air and an agarose film poured as a cylindrical shell inside the tube. Lithium fluoride thermoluminescent detector chips (TLD's) and CR-39 plastic nuclear track detectors (PNTD's) accompanied the tubes to provide radiation dosimetry. The tubes were inoculated with worms and bacterial food, *E. coli K12* strain *χ1666* controlled with kanamycin and streptomycin; nystatin was also included to prevent yeast and fungal contamination. Suspensions of worms in 1 ml of buffer were housed in additional tubes used for radiobiology objectives. The Type I container configuration is shown in Figure 1. On IML-1 the samples were 1) unstowed on-orbit from passive thermal control units (PTCU's) carried in mid-deck lockers, 2) incubated in Biorack or the Spacelab tunnel belt, and 3) restowed in PTCUs for landing.

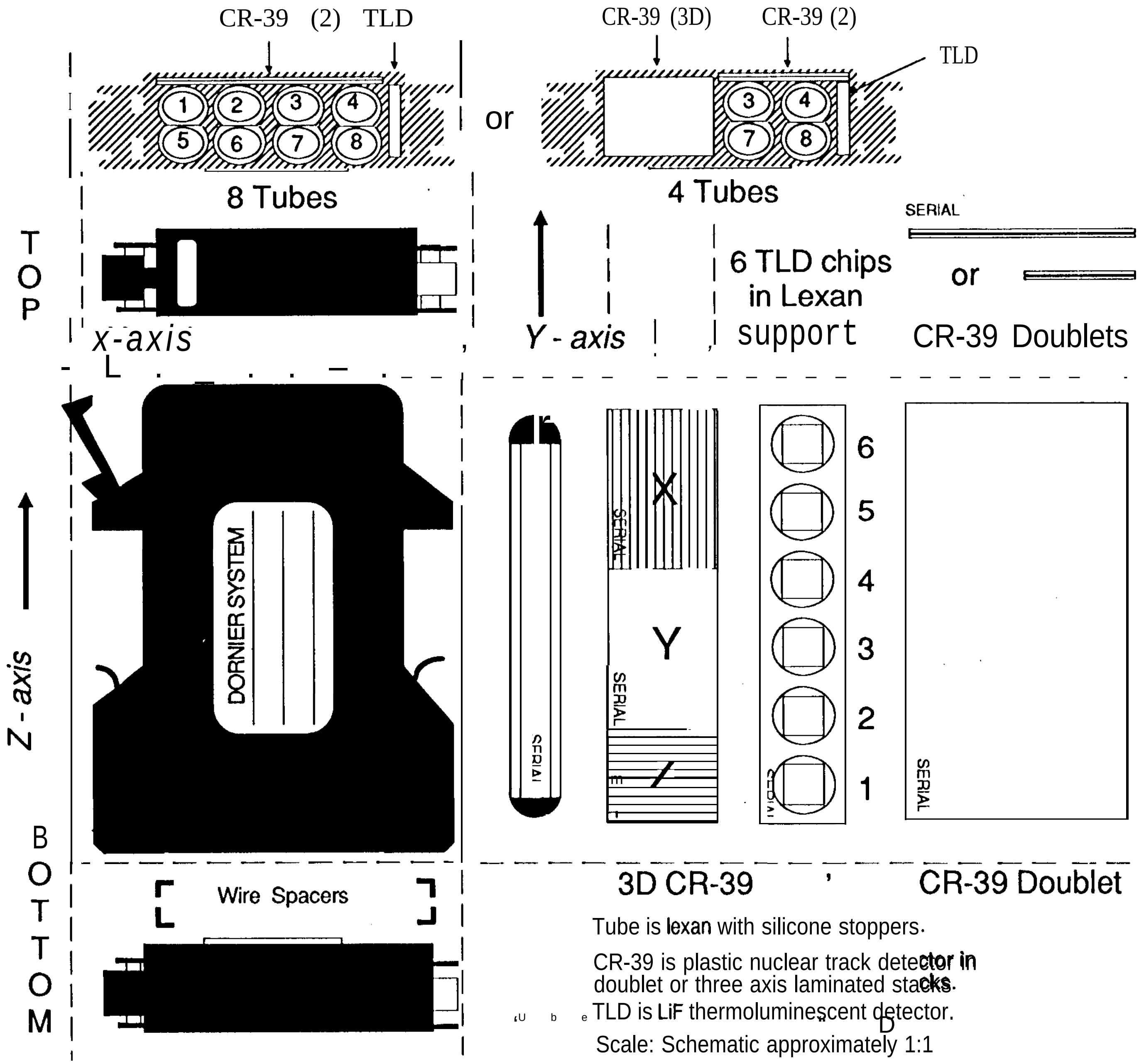


Fig. 1 Configuration of hardware components placed in Biorack Type I containers used in development and chromosome mechanics experiments. Animals were placed in tubes which contained metabolically inactivated bacterial lawns on thin films of agar cast on the inner surfaces of the tubes.

organisms

The subjects were microscopic (1 mm x 60pm diameter) free-living nematodes of the species *Caenorhabditis elegans* whose biology, genetics and handling are reviewed in Wood /3/. C. *elegans* has been studied extensively with respect to development and genetics and is notable in having a fixed cellular anatomy which has been described at the electron microscope level and whose cell lineages have been completely characterized from zygote to adult. The worm has five pairs of autosomes and 1 or 2 X chromosomes defining males (5AA + XO) and self-fertilizing hermaphrodites (5AA + XX), respectively. At 20°C. a hermaphrodite will begin laying -280 eggs 3 days after fertilization. Thus, an 8-day spaceflight holds the potential for 2+ generations. A particular larval form (dauer larva) can survive for several months without feeding in a dormant state. Dauers resume normal development approximately 12 hours after restoration of adequate food.

All strains used for IML-1 were obtained from the *Caenorhabditis* Genetics Center at University of Missouri, Columbia, MO. (currently moving to the University of Minnesota) or were constructed in our laboratory. The wild type strain is N2, variety Bristol, and mutant strains contain the following recessive alleles: *dpy-5(e61)I, unc-13(e51)I, dpy-17(e164)III, dpy-18(e364)III, unc-32(e189)III, unc-5(e53)IV, unc-22(bc96)IV, dpy-11(e224)V, and him-5(e1490)V.* The phenotypes of *dpy* mutants are dumpy, abnormally short and of approximately normal diameter. *Unc* uncoordinated mutants have neurological and muscle defects resulting in partial to complete paralysis or rhythmic "twitching" (uric-22). *Him* high incidence of male mutants are defective in X-chromosome disjunction and produce up to 40% male offspring as compared to the spontaneous rate of O. 15%. Male sperm, transferred during mating, are used preferentially over endogenous hermaphrodite sperm to effectively produce "outcross" progeny.

Experimental Design

The experimental design takes advantage of the invariant developmental timing of the nematode coupled to a genetic strategy which allows chromosome behavior to be followed and different generations to be identified by mutant phenotype. Four tests were carried out, each with two different genotypes to control for specific gene effects. These tests are referred to as: "growth", "mating", "segregation" (of unlinked markers) and "recombination" (plus segregation of linked markers). Worms from selected cultures were fixed and stained using the fluorescent DNA-binding dye DAPI (diamidinophenolindole) to reveal cell nuclei. Some embryos recovered at landing were also fixed and stained with antibodies specific for cytoplasmic determinants that localize to different cell lineages. In every case simultaneous ground controls were performed using sibling animals or identical subcultures in the duplicate Biorack run at NASA's Hanger L near the launch site. Tubes were photographed or videotaped and unloaded for processing at NASA's Dryden Test Flight Facility at Edward's Air Force Base about 6 hours after de-orbit burn signalled the return of gravity to the samples. Six hours represents mid embryogenesis of the worms.

Growth. Six third larval stage (L3) Wild Type or mutant genotype animals were inoculated into tubes incubated in the Spacelab tunnel belt or at 22 degrees C in Biorack incubator A and allowed to grow and reproduce. After recovery population distributions of worms were measured, sets of animals were fixed or frozen for DAP1 and antibody staining. Embryos were also isolated by dissection of gravid hermaphrodites and preserved for antibody staining.

Mating. Six fourth larval stage (L4) males were inoculated with six dauer larvae hermaphrodites Of either *dpy-11* or uric-22 genotype and incubated in Incubator A ± centrifuge. The timing of handover and launch assures that virgin males will accumulate sperm for at least one day prior to mating while hermaphrodites develop into young adults. Successful mating will generate outcross heterozygous $F_1$ animals (50% male) which could engage in a second round of mating to generate Dpy or Unc males in the $F_2$ generation. Phenotypic ratios of animals present at recovery were measured. New progeny were not counted.

Segregation. Six triply heterozygous L3 hermaphrodites were inoculated into tubes and incubated in Incubator A ± centrifuge. Developmental timing assures that fertilization can only commence after orbit is achieved and samples are loaded into incubators. The two genotypes were: uric-5 *IV/+; dpy-11 I him-5 V* or *dpy-18 III/+; uric-l* 7 *IV; him-5 V/+ . The* theoretical Mendelian segregation ratio of 9:3:3:1 should obtain for Dpy and Unc in the $F_1$ generation and ¼ of $F_1$s should also be *him-5/him-5.* Males will only appear at the spontaneous rate of O. 15% in the $F_1$. The $F_1$ Him animals will generate up to 40% males in their broods. Therefore the appearance of Wild Type, Dpy, Unc and Dpy Unc males is due to $F_2$'s. The phenotypic ratios of males present at recovery were measured.

Recombination. Six triply heterozygous L3 hermaphrodites with linked *dpy* and unc genes were inoculated into tubes and incubated in Incubator A ± centrifuge. The two genotypes were: *dpy-5 unc-13 I/+ +; him-5 V/+* and *unc-*32 *dpy-17 111/ + +; him-5 V/ +. The* theoretical Mendelian segregation ratio of 3:1 should obtain for WT and Dpy Unc in the $F_1$ generation along with Dpy and Unc recombinant at 1.8 and 2.6 % for the two strains, respectively. One fourth of each $F_1$ genotype should also be *him-5/him-5.* As with the "Segregation" test males will be $F_2$'s. Hermaphrodite and male animals present at recovery were scored.

It is important to note that in a typical laboratory experiment it is possible to rigorously separate generations by moving animals regularly to separate petri dishes before they reproduce. However, in the flight experiment, no human intervention occurred for two generations, so that the final population is a mixture of three generations. It should also be noted that the growth rates and fertilities of the mutant phenotypes are reduced relative to the Wild Type so that systematic distortions to the theoretical Mendelian ratios will occur if animals are scored at any given time point. Flight, ground, and centrifuge controls are therefore critical.

## PRELIMINARY RESULTS AND DISCUSSION

The overall impression from preliminary analyses of IML- 1 samples is that nematode development and chromosome mechanics are not significantly affected by gravity unloading. Despite classical observations on *Ascaris* embryos showing that substantial hypergravity can perturb nematode development it is not unexpected that small, rapidly developing embryos and larvae would function well because they are below a size and mass threshold where gravity is expected to be important. Malacinski /4/ has reviewed this problem and the difficulties in interpreting data of the form "normal" or "abnormal" with respect to morphology. C. *elegans* has advantages over many other systems in this respect because of the near complete invariance between individuals. Nevertheless we are continuing to build up a statistical base to support the tentative "normal" conclusion.

Growth. Vigorous growth and reproduction occurred resulting in final populations of from 600 to 7320 worms per tube averaging 3977 ± 1266 worms for the 32 tubes incubated at 22 degrees C. In some cases the animals had just exhausted their food supply at recovery but newly laid eggs were present in the cultures and animals were active indicating that hertnaphrodites were not behaviorly affected by food restriction. No obvious pattern in the population size vs incubation condition was detected that was independent of genotype, A slight trend towards larger population size was observed for tubes incubated on centrifuges in space or on the ground. One may speculate that slight vibration induces more movement and feeding of sluggish mutant animals.

Mating. Mating of *C. elegans* is often erratic even under the best of laboratory conditions. One round of mating was efficient in Space leading to many outcross progeny and a few $F_2$ mutant males were also present indicating that a second round of mating between $F_1$ animals also occurred. Thus the most complex behavior of worms appears to be insignificantly perturbed by microgravity in this hardware configuration. The intrinsic variability prevents a more quantitative assessment.

Segregation. $F_1$ and $F_2$ progeny were produced by heterozygous hermaphrodites in proportions which were not significantly different between controls and test samples. The proportions of $F_2$ males deviated from the theoretical 9:3:3:1 Mendelian ratio but this can be accounted for by different growth rates and fertilities of mutants. As only animals present at recovery were scored (new progeny were ignored) these numbers also represent broods from hermaphrodites whose reproduction was interrupted before completion.

Recombination. As with segregation, $F_1$ and $F_2$ progeny were produced by heterozygous hermaphrodites in proportions which were not significantly different between controls and test samples. The proportions of $F_2$ males deviated from the theoretical 3:1 Mendelian ratio, but again this can be accounted for by different growth rates and fertilities of mutants in the mixed generation culture scored at a single time period. Many $F_1$ recombinant hermaphrodites were seen and a few $F_2$ males were present, Table 1 illustrates the segregation pattern of offspring from one experiment in which tubes were inoculated with hertnaphrodites of genotype *dpy-17* uric-32 *III/* + +; *him-5/* + *V.* In this experiment males are all $F_2$ individuals whereas hermaphrodites are the sum of $F_1$ and $F_2$ offspring present at the time of recovery at Edwards AFB. The expected proportions of phenotypes (uncorrected for growth rate and single sampling time) are approximately 3:1 for Wild Type: Dpy-17 Uric-32 offspring, Dpy-17 Non-Unc-32 and Uric-32 Non-Dpy-17 animals are recombinant individuals from crossover events between uric-32 and *dpy-17* which are separated by 3 centimorgans.

TABLE 1. Segregation and Recombination of Linked Marker Mutations.
Phenotypic Composition of $F_1$+$F_2$ Hermaphrodites and $F_2$ Males.

| G-Level Fl or Gr* | Wild Type Herm. | Wild Type Males | Dumpy-Uncoord. Herm. | Dumpy-Uncoord. Males | Dumpy Herm. | Dumpy Males | Uncoordinated Herm. | Uncoordinated Males |
|---|---|---|---|---|---|---|---|---|
| O Fl | 1839 | 56 | 493 | 1 | 25 | 1 | 23 | 1 |
| 1 Fl | 2135 | 103 | ·383 | 7 | 23 | 3 | 77 | 6 |
| 1 Gr | 889 | 29 | 60 | 0 | 15 | 4 | 27 | 0 |
| 1.4 Gr | 1186 | 55 | 172 | 1 | 18 | 2 | 24 | 0 |

•Fl and Gr refer to flight and ground samples, respectively.

Anatomical Observations. About 100 animals have been analyzed to date for their anatomy based on cell number and distribution, nuclear morphology, karyotype and symmetry relations. Particular emphasis was given to the gonad vs intestine symmetry /5/ relationship which derives from early (6-cell stage) cell division planes and to the linear gametogenesis sequence of nuclei in the gonad /6/. No obvious differences have been seen between flight and ground Wild Type animals and no alterations in gonad symmetry were detected with the initial limited sample. No defective karyotypes have been seen and the only unusual feature detected is the presence of intestinal cells with incomplete nuclear divisions (anaphase bridges) in both flight and ground samples.

Observations on a small number of late (at least comma stage) embryos fixed at recovery (about 6 hours after de-orbit burn restores gravity levels) show no obvious disruptions to anatomy. The timing of development and mission operations is such that post comma stage embryos at recovery were all fertilized and passed through critical developmental events prior to deorbit burn. Young embryos resulted from zygotes which experienced reentry accelerations. In these embryos cellular anatomy was also normal and synchronized mitoses were observed suggesting no significant disturbances to cell cycles. The question of maternal spatial patterning in embryos has been addressed using maternal effect embryonic lethal mutations and immunofluorescence of microtubules, actin, P-granules and membrane antigens. In all cases the evidence points to a homogeneous distribution of cytoplasm in oocytes. The first event establishing polarity is sperm entry at the surface of the oocyte which first enters the spermatheca (valve connecting oviduct and uterus). This site becomes the posterior pole of the embryo but it is not known whether the anterior-posterior axis exists before sperm entry, Dorso-ventral and left-right axes are not evident until at least the 4-cell stage /8/. Antibodies directed against germline "P-granules" and the myosin expressed in the pharynx reveal normal staining patterns suggesting normal distribution of embryonic cytoplasmic determinants

/3/. P granule segregation has been shown to be controlled by microfilament activity in a specific 10 minute interval of the first cell cycle following fertilization. Only the P4 cell in young IML- 1 embryos and the Z2 & Z3 germline cells of mature embryos and young larvae contained granules as expected /8/. Pharyngeal myosin was expressed midway through development and its normal structure requires embryonic induction events as well as cell autonomous activities at the 6 to 24-cell stage leading to final development. These observations and other data reduction activities are continuing.

## SUMMARY

Based on preliminary observations there are no obvious differences in the development, behavior and chromosome mechanics of C. *elegans* as a function of gravity unloading. The animals successfully reproduced twice in space with the generation of many thousands of offspring. Both self-fertilization and mating of males with hermaphrodites was successful. Gross anatomy, symmetry and gametogenesis were normal for a small sample set based on light microscope observations. No defective karyotypes or cell distributions were observed. Finally, the pairing, disjoining and recombination of chromosomes showed no differences correlated with gravity levels, This fact lends further support to radiobiology experiment interpretations referenced to radiation levels alone. Total doses to samples of 0.80 to 1.10 mGy were not expected to perturb development in any significant way.

## ACKNOWLEDGEMENT

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